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ORIGINAL PAPER

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Affective disorder subtyped by psychomotor symptoms, monoamine oxidase, melatonin and cortisol: identification of patients with latent bipolar disorder

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Abstract A multivariate approach using pattern recognition method was applied on a multivariable data set from patients with affective disorders comprising biological and clinical variables. The depressed patients were rated according to 23 items of the comprehensive psychopathological rating scale (CPRS). Variables of importance were selected and clusters of patients were found by combining monoamine oxidase, melatonin and post-dexamethasone cortisol with symptoms of psychomotor retardation and agitation. Patients were distributed with high scores of agitation in the extreme of one direction and with high scores of retardation in the opposite direction. By using the combined clinical and biological variables, a diagnostic subcategory with latent bipolar disorder was identified. Two clusters of unipolar patients, one with low melatonin and low psychomotor retardation scores, and one with high melatonin and high psychomotor retardation scores, were found. Identification of a patient group with latent bipolar disorder may have potential therapeutic value since bipolar patients should be taken care of by a specialist in psychiatry, avoid tricyclic antidepressant therapy and may be candidates for lithium treatment.

Key words Classification · Affective disorders · Depression · Bipolar disorder · Monoamine oxidase · Cortisol · Melatonin · Principal component

Introduction

The operational criteria for the diagnosis depression is based on categorical and/or quantitative variables. A subtype of depression, affective psychosis, is characterised by genetic vulnerability and a positive clinical treatment outcome (Zimmerman and Spitzer 1989; Post 1992). In patients suffering from affective psychosis, abnormal dexamethasone suppression is observed in half of the individuals as a biological sign of neuroendocrine dysfunction (WHO 1987). Patients with major depression have been suggested to have serotoninergic and/or noradrenergic dysregulation (Schildkraut 1965; Siever and Davis 1985; Harro and Oreland 1996; van Praag 1996). A dysfunction of these neurotransmitters may be caused by environmental stressors, which often increase the adrenal cortisol production and disturb the feedback regulation of the hypothalamic–pituitary–adrenal (HPA) axis (Dinan 1996). These disturbances in the regulatory functions of the central nervous system may be detected through endocrine, as well as biochemical and other laboratory measures. In a previous study, results of platelet monoamine oxidase (MAO) activity, serum melatonin and serum cortisol, following the dexamethasone suppression test, indicated the presence of at least two biologically characterised groups of depressed patients (Wahlund et al. 1995). The biological variables in the previously reported sample were statistically analysed with multivariate methods, together with the scores of rated clinical symptoms. In the present report we aim to further evaluate and describe the features of subpopulations in affective disorders.

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Subjects and methods

Clinical assessments

There were 28 acutely ill hospitalised patients (17 women and 11 men), consecutively admitted during the years 1980–1982 to the Department of Psychiatry at St. Göran's Hospital, Stockholm, with a major depressive disorder diagnosed according to Research Diagnostic Criteria (RDC; Spitzer et al. 1978). The diagnosed depression was of unipolar type in all subjects. Patients with alcohol or drug dependency, co-morbidity of schizophrenia and severe somatic illness, in particular endocrine diseases, were excluded. The mean age ± standard deviation (SD) was 42 ± 10 years with a range of 26–63 years. In the statistical calculations the gender was coded as female = 1, and male = 2 (see Fig. 1c).

Table 1 Results of platelet monoamine oxidase *(MAO)* activity measured in nanomoles phenlethylamine formed/milligram protein per minute, melatonin in serum as nanomoles per litre and post-dexamethasone serum cortisol *(Cpdx)* as nanomoles per litre, and the scores of the clinically rated psychomotor symptoms Reduced speech and Slowness of movement in 28 patients diagnosed as unipolar depression in 1980–1982. Patients 3, 5, 7, 16 and 19 were later hospitalised for mood disorder of bipolar type which became apparent in a 15-year follow-up study

Patient no.	MAO	Melatonin	Cpdx	Reduced speech	Slowness of movement
1	1.18	0.21	828	0.00	0.50
2	0.66	0.21	208	1.25	0.75
3	2.82	0.21	724	0.25	1.00
4	0.19	0.19	120	0.00	0.50
5	1.11	0.24	330	0.50	0.50
6	0.97	0.55	88	0.00	0.00
7	1.70	0.21	337	0.00	1.50
8	1.01	0.23	29	0.75	0.50
9	0.13	0.18	25	0.00	0.00
10	0.72	0.20	501	0.75	0.25
11	1.25	0.39	41	0.50	0.50
12	0.49	0.18	535	0.25	0.00
13	1.06	0.77	159	0.00	0.00
14	1.45	0.08	131	0.75	0.00
15	0.44	0.10	106	0.00	0.25
16	1.03	0.37	466	0.00	0.00
17	0.98	0.37	198	0.75	0.75
18	1.13	0.13	340	0.00	0.00
19	1.58	0.18	307	0.75	0.75
20	0.95	0.26	38	0.50	0.75
21	0.72	0.11	556	0.50	0.00
22	1.14	0.27	577	0.25	1.00
23	1.14	0.14	410	0.00	0.00
24	1.77	0.41	33	1.50	0.75
25	0.63	0.12	508	2.25	2.00
26	1.04	0.35	100	0.50	0.50
27	0.65	0.26	107	0.75	0.75
28	0.53	0.21	100	0.00	0.00

Table 2 Principal component analysis of the 23 Comprehensive Psychopathological Rating Scale *(CPRS)* items examined in 28 depressed patients. Loadings of the two components (PC1 and PC2) and mean ± SD of clinically rated scores of each CPRS item are shown. (From Beck-Friis et al. 1985)

Item no.	CPRS item	PC1	PC2	Mean ± SD of rated scores
Reported:				
1	Sadness	0.34	–0.12	1.58 ± 0.62
3	Inner tension	0.14	–0.04	1.41 ± 0.51
5	Inability to feel	0.20	0.17	1.49 ± 0.64
6	Pessimistic thoughts	0.30	0.12	1.44 ± 0.55
7	Suicidal thoughts	0.24	0.13	1.04 ± 0.64
8	Hypochondriasis	–0.15	0.22	0.32 ± 0.65
9	Worrying over trifles	0.20	–0.11	0.91 ± 0.70
13	Indecision	0.27	–0.06	1.25 ± 0.66
14	Lassitude	0.32	–0.04	1.41 ± 0.69
15	Fatiguability	0.24	0.18	0.99 ± 0.80
16	Concentration difficulties	0.28	0.03	1.34 ± 0.58
17	Failing memory	0.11	0.23	0.74 ± 0.62
18	Reduced appetite	0.14	0.11	0.86 ± 0.74
19	Reduced sleep	0.12	0.42	1.40 ± 0.90
24	Aches and pains	0.05	0.43	0.44 ± 0.63
25	Muscular tension	–0.03	0.22	0.68 ± 0.73
Observed:				
41	Apparent sadness	0.27	–0.01	1.51 ± 0.52
54	Reduced speech	0.12	–0.27	0.45 ± 0.54
58	Perseveration	–0.16	0.06	0.14 ± 0.43
60	Slowness of movement	–0.07	–0.35	0.48 ± 0.50
61	Agitation	0.15	0.40	0.42 ± 0.51
63	Muscular tension	0.00	0.37	0.70 ± 0.59
66	Global rating of illness	0.33	0.06	1.86 ± 0.61
Eigenvalues of the first two components		6.91	3.05	

Follow-up diagnoses

The 28 patients with affective disorders were all of the unipolar type when first investigated during the years 1980–1982 (Beck-Friis 1983). When the patients' medical records and research protocols were examined 15 years later for evaluation of the follow-up diagnosis, however, 5 patients had experienced symptoms of at least one manic or hypomanic episode, severe enough to require hospital care. These 5 patients were now diagnosed under code F31 (Perturbation affectiva bipolaris) according to ICD-10 (WHO 1993), and as suffering from bipolar disorder according to DSM-IV diagnostic system (American Psychiatric Association 1994). At follow-up, the remaining 23 patients fulfilled the criteria for unipolar depression according to ICD-10 and DSM-IV.

Clinical ratings

Clinical symptoms were rated for all subjects by two experienced psychiatrists on the first morning of the study using the Comprehensive Psychopathological Rating Scale (CPRS) including 22 items for depression (Åsberg et al. 1978). The CPRS items were scored by definition from 0 to 3 using half points, with 0 indicating the absence of particular symptoms and 3 indicating the most severe degree. The mean of the scores of the raters was used for each of the 22 CPRS items, as well as for the additional item, CPRS global score (CPRS-GL), which rates the overall severity of depression. The raters' scores agreed satisfactorily for all but 1 item (concentration difficulties; Beck-Friis 1983, p. 38). The individually rated raw scores of the 2 items specifically used for statistical clustering in the present investigation, i.e. items Reduced speech, and Slowness of movement, are shown in Table 1. The remaining 21 items, which are sensitive for depressive symptoms, are shown in Table 2.

Medication

Sixteen of the 28 patients were without medication for at least 1 month prior to and during the clinical and laboratory investigations. No washout period was instituted. The patients who had been prescribed medication maintained the same dosage as before hospitalisation to avoid possible withdrawal effects (Ross et al. 1980; Charney et al. 1982; Dilsaver et al. 1983). Six patients were prescribed tri- or tetracyclic antidepressives and/or lithium medication, 3 patients were on lithium and a low dose of benzodi-

azepine. Alimemazine, benzodiazepine or propriomazine were prescribed in low doses as sedatives when needed.

Laboratory investigations

Prior to laboratory investigation, all subjects were clinically evaluated and their psychiatric symptoms were rated. The mean duration stay prior to the laboratory testing was approximately 2–3 days. If not otherwise mentioned, all laboratory assays were determined in duplicate or triplicate.

Monoamine oxidase

Platelet monoamine oxidase (MAO) activity was determined with phenethylamine as substrate at a final concentration of 1.25 µm. Activity is expressed as nanomoles product formed/milligram protein per minute (Koide et al. 1981). Raw data are presented in Table 1.

Melatonin

Serum melatonin was determined at ten time points over a 24-h period beginning at 08 h. Maximum nocturnal levels were used in the calculations. Serum melatonin was analysed by the radioimmunoassay method described by Wetterberg et al. (1978). The lower limit of detection was 0.01 nmol/l and the percentage variation (CV%) 6.8 (intra-assay variation 7.4% and inter-assay variation 4.8% for samples above 0.15 nmol/l). Serum melatonin was expressed as nanomoles per liter. The melatonin data shown in Table 1 have been adjusted for body height, according to Beck-Friis et al. (1984).

Maximum post-dexamethasone cortisol

The dexamethasone suppression test (DST) was performed with oral administration of 1 mg dexamethasone at 22:00. Serum cortisol samples were drawn at 08:00, 16:00 and 22:00 on the following day ("post dexamethasone"). The maximum post dexamethasone concentration of cortisol for any of the three time points was used in the present study. Serum cortisol was analysed using a commercially available RIA kit (Famos Diagnostica, Turku, Finland). The intra-assay coefficient of variation for serum cortisol was below 3% and the inter-assay coefficient of variation was 5% for samples within the normal range (n = 20). The concentration of serum cortisol was expressed as nanomoles per liter. Raw data are seen in Table 1.

Statistics and data analysis

Principal component analysis

In order to examine common properties among individuals, measured as symptoms and biological variables, principal component analysis (PCA) was used. Principal component analysis finds the linear combination of the original variables which identify the variables with most variance. The number of components may be decided by cross validation or by cumulated variance (Wold 1978; Jolliffe 1986).

The distribution of individuals can be presented as a diagram by plotting the scores of principle component 1 vs scores of principle component 2. The interpretation of the analysis is facilitated by scattergrams of the results.

To detect clusters and patterns a non-parametric density estimation was applied to the scores (Bowman and Foster 1992). The non-parametric density estimation procedure was performed with JMP (Lehman et al. 1995), in which the plot of scores are accompanied by a corresponding density estimation plot as well as a loading plot. The loading plot contains information on how variables may be correlated (Gabriel 1982; Geladi and Kowalski 1986).

Construction of a summarised principle component (PC) loading of random variables

The selection method used in this study was based on the observation that variables with low loadings are of less importance for clustering when the first components are significantly informative according to cross-validation criteria or when they capture most of the variance. Exclusion of variables may be performed by a mean loading vector of sampled normal random variables. Variables with loadings less than the cut-off radius in the plane of the projected components, defined by the mean random loading vector, are taken as loadings based at random.

The equation describing the cut-off procedure for the level of significance of the random loading variables of two principal components was performed with the following requirements:

1. The number of random variables had to be equal to the number of experimental variables in the data matrix.
2. The random variables were standardised.
3. Principal component analysis of the two first principal components had to be performed.

A mean random vector ($\bar{\rho}_2$) is based on the loadings of the first and second principal components (p_{i1} and p_{i2}). The denominator k denotes the numbers of the examined variables in the sample. The length ($|\bar{\rho}_2|$) of the mean random vector is determined by the following equation:

$$|\bar{\rho}_2| = \frac{+\sqrt{\sum_{i=1}^{k}(p_{1i}{}^2 + p_{2i}{}^2)}}{k}.$$

Computer programs

All statistical and mathematical calculations including principal components were performed by JMP version 3.1 (Lehman et al. 1995) developed by SAS Institute (Statistical Analysis Systems). Visual analysis of data was performed on JMP. Calculations of principal components was also performed by SIMCA-S 6.01 (Umetri 1997).

Written informed consent was obtained from all individuals before participation. The investigation was approved by the ethics committee of Karolinska Institute.

Results

Principal component analysis of clinical items

Principal component analysis was performed on 23 CPRS items as well as gender (Fig. 1). Individuals were grouped according to the items of the core symptoms for depression and for gender (Fig. 1). The cut-off criteria were based on the mean of loadings of 24 random variables, because the mean random loadings change with the number of measured variables.

Clinical CPRS symptom ratings

Three types of clinically related symptoms were found close together in the loading plot and used for further statistical analysis (Fig. 1 c). The first two components were significant according to cross validation. Variables with the highest loading of their cut-off criteria were selected for further

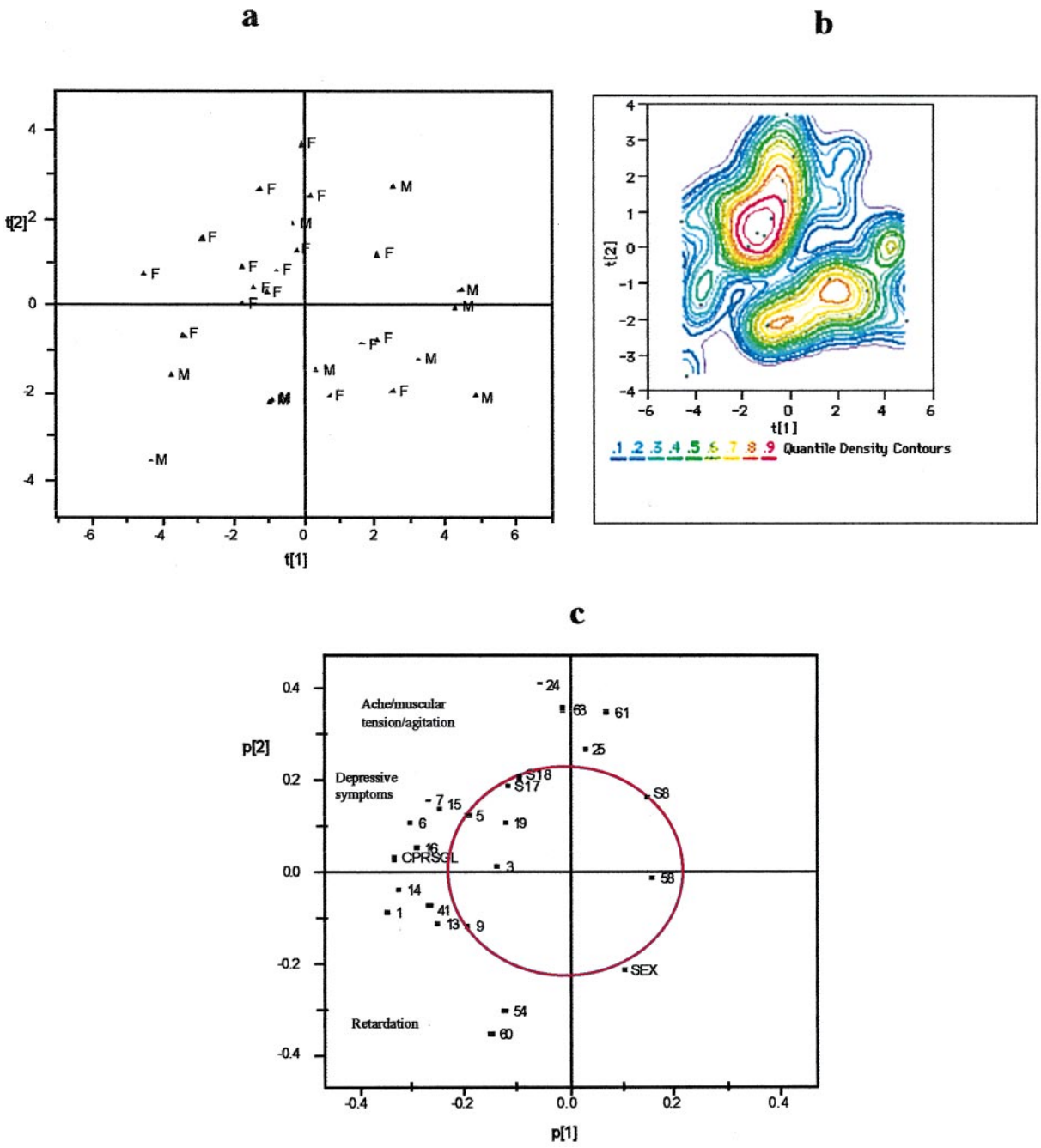


Fig. 1 a–c Representation of principle component (PC) analysis of the scores of 23 CPRS items and gender of 28 patients. **a** A score plot of individuals; **b** a topographic density plot; **c** a loading plot with the 24 examined variables. Two clusters were identified (**b**). The individuals disjoined according to gender. Female patients had more severe depressive symptoms. Items within the circle in **c** were at random. Three groups of CPRS-rated symptoms are shown in the text and are also indicated by the *arrows* in **c**. Anxiety items and retardation items are independent of gender, whereas the depressive symptoms are not. The scale of axes in **a** and **b** is z-scores (*t*). The diagram in **c** is composed of the coordinates of the cosine angles of the first two PC vectors (*p*). *F* Female; *m* male

analysis. The CPRS items 24, 25, 54, 60, 61, 63 (see Table 2) loaded perpendicular to items frequently present in depression. Some items with high loading were intercorrelated and grouped together in the loading plot as shown in Fig. 1 c.

The three main groups in the calculations were classified as follows:

1. Group 1 contained items of typical depressive symptoms, rated by CPRS, with item numbers 1, 5–7, 13–15, 41, and the global rating score (CPRS-GL) which is a separate item of the scale (see Table 2).
2. Group 2 contained symptoms of anxiety, i.e. agitation, muscular tension and ache and pain, rated as CPRS item numbers 24, 25, 61 and 63.

3. Group 3 contained retardation symptoms, i.e. reduced speech and slowness of movements, CPRS item numbers 54 and 60.

Item numbers 3, 5, 8, 9, 17–19 and 58 were below the cut-off criteria and did not contribute in the loading of clusters.

PCA on selected clinical items and the biological variables

The individual data of the clinical scores and the biological variables used for PCA are listed in Table 1. The PCA was performed on groups 1, 2 and 3, which contained scored clinical symptoms (Fig. 1c), together with the three biological variables, platelet monoamine oxidase (MAO) serum melatonin and post-dexamethasone serum cortisol. Using PCA, developed for the specific purpose of allowing scores of rated symptoms and laboratory variables to be handled together, four clusters of depressed patients were found (Fig. 2a, b). The PC loadings of the psychomotor retardation symptoms giving the highest loadings "Reduced speech" and "Slowness of movements" are shown in Fig. 2c, together with the PC loadings of platelet MAO activity, serum melatonin and post-dexamethasone serum cortisol. The patients disjoined along a psychomotor axis, with retardation symptom at one extreme and the agitation symptom at the opposite end, as shown in Fig. 2d. Age, medication, height and weight were also examined together with the clinical and biological variables but did not contribute to the clustering of individuals in the plane of the first two principal components.

Subpopulations of patients with affective disorders

The 5 patients who developed bipolar symptoms during the follow-up period and had significantly higher MAO activity than the unipolar patients were gathered mainly into one of the clusters (cluster III in Fig. 2a). However, the MAO activities alone were not sufficient to cluster the bipolar patients. As is seen in Table 3, there was also a significant difference in melatonin between the bipolar patients and the two subgroups of depressives who were still diagnosed as unipolar in the follow-up study, 15 years after the laboratory testing was performed. Upon entry into the study in 1980–1982, the five bipolar patients were clinically "latent carriers" of the trait for bipolar illness, in the sense that they had not, during that period of time, experienced any hypomanic or manic episodes. At different times during the 15 year observation period, the five latent bipolar patients became patients with clinically manifest bipolar illness.

It might be expected that up to 7 of the 28 patients (25%), diagnosed as unipolar depressives when first investigated would later experience bipolar symptoms (Angst 1996). Further follow-up studies of the unipolar patients will tell if the additional 2 patients are expected to be carriers of the trait for bipolar illness belong to cluster III. There were no bipolar patients found among the 15 unipolar individuals found in clusters I and II (Fig. 2a).

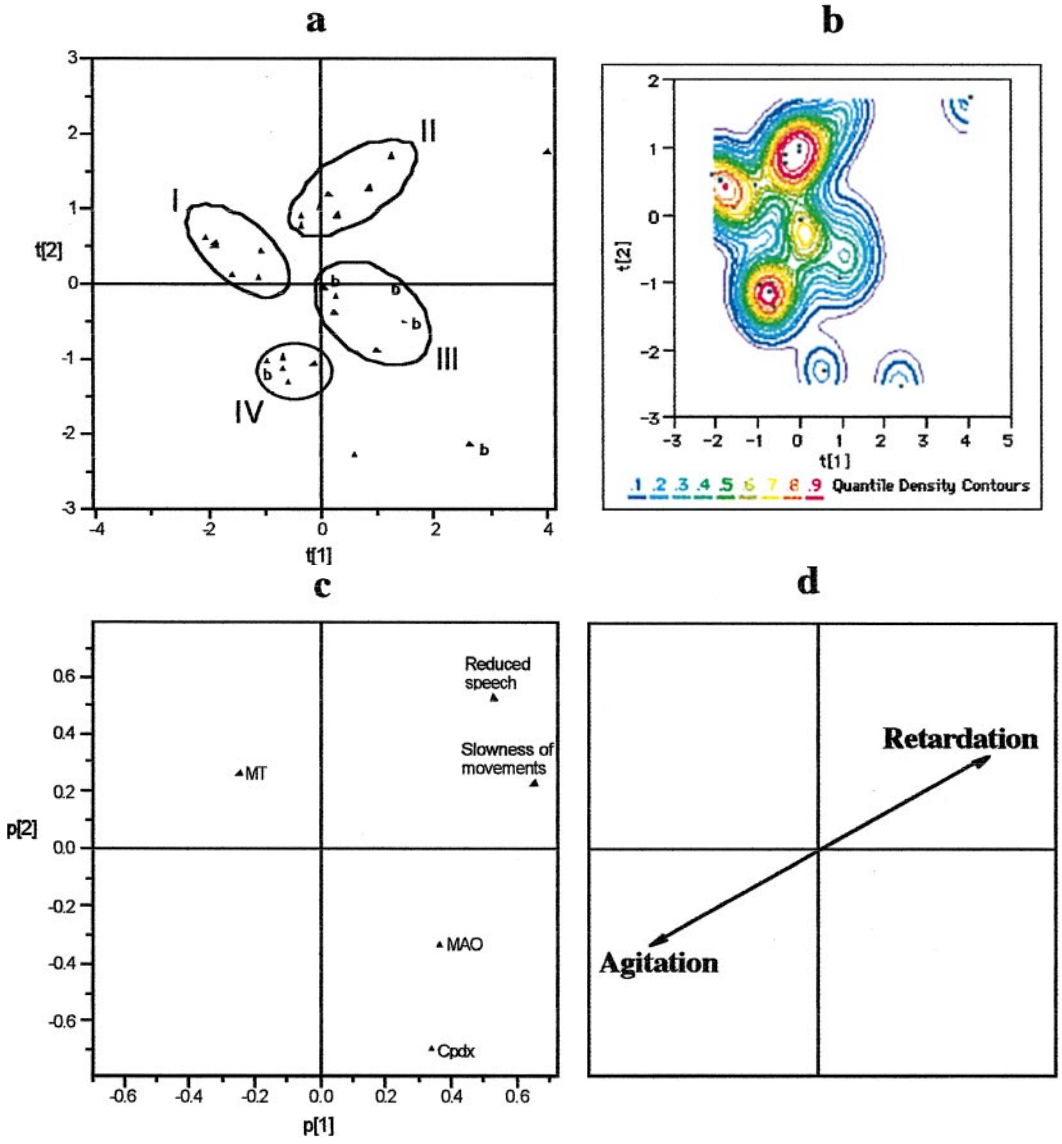
When testing the sample for clustering, without the individuals in cluster III, and the one bipolar patient in cluster IV, 20 unipolar patients remained (clusters I, II and IV in Fig. 2a). In the second clustering process of the 20 unipolar patients two new clusters were found, one with 9 and one with 11 patients in each cluster (Fig. 3). The two new clusters of unipolar patients (Uni I and Uni II) were compared with the bipolar group, as well as between themselves, for statistical differences in the tested variables (Table 3). The Uni-I group differed from the bipolar group, not only in MAO, but also in melatonin and the psychomotor symptom Slowness of movement. The Uni-II group of patients differed from the bipolar group in MAO, melatonin and post-dexamethasone cortisol, but not in the CPRS-rated psychomotor symptoms.

The two unipolar patient groups, Uni I and Uni II, differed markedly in maximal serum melatonin concentration at nighttime with a mean of 0.16 nmol/l for Uni I, and 0.36 nmol/l for Uni II. The five latent bipolar patients had a mean for melatonin of 0.24 nmol/l, a value approximately half that between the two unipolar subgroups. Furthermore, there was a difference in the degree of the two rated psychomotor symptoms, with significantly lower-rated scores for the items Reduced speech and Slowness of movement, in the patient group Uni I, which also had significantly lower serum melatonin.

Discussion

In this study we used an approach with the potential of treating discrete scoring, continuous variables, and biological variables in one and the same statistical equation. An interpretation of the finding along the psychomotor axis is that retardation and agitation are not mutually exclusive, but rather vary continuously along the axis describing different degrees of psychomotor expression and that agitation may be partly related to the degree of anxiety present. More significantly, the analysis found that patients clustered in the area defined by the intersection of a vertical projection of a symptom axis composed of these psychomotor items, and a second, perpendicular axis, composed of MAO activity, serum melatonin and post-dexamethasone cortisol levels.

Statistical evidence in this study supports the hypothesis that carriers of latent bipolar disease trait are characterised by a cluster of clinical and biochemical variables, which conceivably might have identified this population at the time of the first depressive episode. If this finding of possible predictors of latent bipolar traits is replicated in other studies, the result could have considerable therapeutic implications. Patients with bipolar illness should be treated by a specialist and tricyclic antidepressants should not be prescribed, since they may precipitate manic mood episodes (Zis et al. 1979). In pa-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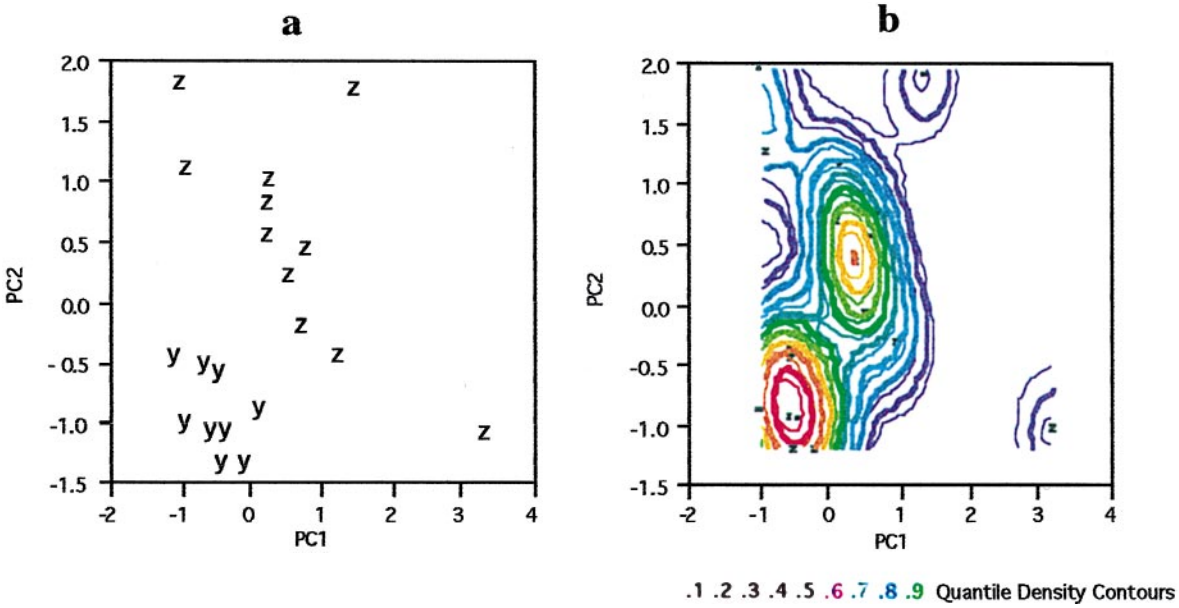
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3

Table 3a Patients in different diagnostic categories according to statistical clustering as seen in Fig. 2. Mean ± SD of platelet monoamine oxidase *(MAO)* activity measured in nanomoles phenlethylamine formed/milligram protein per minute, melatonin in sereum s nanomols, post-dexamethasone serum cortisol *(Cpdx)* as nanomoles per litre, and the retaed psychomotor symptoms (CPRS) Reduced speech and slowness of movement in 28 patients diagnosed as unipolar depression in 1980–1982. In a follow-up study, five unipolar patients were later hospitalised for mood disorder of the bipolar type. The remaining 20 unipolar patients in clusters I, II and IV (see Fig. 2) were further tested for statistical clustering into two separate groups

Diagnosis of patient groups	MAO	Melatonin	Cpdx	Reduced speech	Slowness of movement
Bipolar (n = 5)	1.65 ± 0.71	0.24 ± 0.07	432 ± 174	0.30 ± 0.33	0.75 ± 0.56
Unipolar (n = 23)	0.88 ± 0.39	0.26 ± 0.16	249 ± 230	0.49 ± 0.58	0.42 ± 0.48
Unipolar (n = 9)[a]	0.66 ± 0.41	0.16 ± 0.04	336 ± 271	0.08 ± 0.18	0.14 ± 0.22
Unipolar (n = 11)[b]	1.00 ± 0.32	0.36 ± 0.18	137 ± 139	0.80 ± 0.66	0.66 ± 0.53

[a] Cluster I in Fig. 3
[b] Cluster II in Fig. 3

Table 3b The mean ± SD of the results in Table 3a were tested for statistical differences. Degree of significance is indicated. The bipolar group *(Bi)* was compared with the unipolar *(Uni)* groups, as well as with the unipolar subgroups, Uni I and Uni II (see Fig. 3). n.s. non-significant according to the Wilcoxon rank-sum test

Groups tested for differences	MAO	Melatonin	Cpdx	Reduced speech	Slowness of movement
Bi vs Uni (n = 23)	< 0.05	n.s.	n.s.	n.s.	n.s.
Bi vs Uni I (n = 9)	< 0.05	< 0.05	n.s.	n.s.	< 0.05
Bi vs Uni II (n = 11)	< 0.05	< 0.05	< 0.01	n.s.	n.s.
Uni I vs Uni II	n.s.	< 0.01	n.s.	< 0.01	< 0.01

tients with vulnerability to develop bipolar disorders, lithium treatment might be considered, both as protective medication and as treatment during the depressive stage of the disease episode.

It is not clear whether psychomotor disturbances occur less frequently in subpopulations of unipolar depressed patients, although such disturbances have been recognised as features of psychotic depression (Kay et al. 1969, Mendels and Cochrane 1968). Four of five bipolar depressed patients in the present study were characterised by high scores of psychomotor retardation. Sobin and Sackheim (1997) suggest in a review that whether a patient is psychomotor retarded or agitated may depend on whether the illness is unipolar or bipolar. It is generally considered difficult to predict whether patients with a few episodes of Major Depressive Disorder will ultimately evolve into patients with Bipolar Disorder. In DSM-IV it is suggested that an acute onset of depression with psychomotor retardation is likely to predict a bipolar course (American Psychiatric Association 1994, p. 342).

The confounding effect of including latent bipolar patients in studies on depression is inherent in all studies where presumably unipolar patients are selected during the first couple of depressive episodes and cannot be avoided at present. Sufficient observation time is needed to be able to detect bipolar patients hidden among the unipolar depressives. In the present study the clustering of more homogeneous subgroups of unipolar patients was possible because the bipolar patients were identified and treated as a separate subpopulation.

Approximately half of depressive patients displayed increased post-dexamethasone cortisol (Feinberg and Carroll 1984; Holsboer et al. 1984; Maes et al. 1987; Rush et al. 1996). The two clusters of unipolar depressives (see Fig. 3) were only partly determined by post-dexamethasone cortisol levels. Serum melatonin and platelet MAO also contributed to the clustering.

Interestingly, the unipolar depressed patients in subgroup Uni I (Table 3) with the lowest serum melatonin also displayed the lowest platelet MAO activity. This is unexpected since in animal experiments systemic administration of a selective monoamine oxidase-A (MAO-A) inhibitor stimulated melatonin biosynthesis, suggesting that a decrease in MAO activity should increase melatonin production if other regulatory mechanisms are not involved (Oxenkrug et al. 1994).

◀ **Fig. 2a–d** The results of a PC analysis on selected biological variables and rated clinical symptoms. **a** Four main clusters of patients, clusters I, II, III and IV, are shown. Three patients were outliers. The PC loading of the three biological variables monoamine oxidase (*MAO*), melatonin and post-dexamethasone cortisol, and the two clinical CPRS-rated symptoms, Slowness of movements and Reduced speech, are shown in **c**. The scale of axes **a** and **b** is z-scores (*t*). The diagram in **c** is composed of the coordinates of the cosine angles of the first two PC vectors (*p*). An illustration of how individuals disjoined along a psychomotor axis, with retardation symptoms in the upper right corner at one extreme and the agitation at the opposite end in the lower left corner, is shown in **d**. *Cpdx* Post-dexamethasone serum cortisol

Fig. 3 The first two PCs (*PC1* and *PC2*) of the PC analysis on the selected biological variables, monoamine oxidase, melatonin and post-dexamethasone cortisol, and the rated clinical psychomotor symptoms, Slowness of movement and Reduced speech in 20 unipolar depressed patients. Two main clusters of patients were found. The individuals in the two clusters are indicated with *y* for the group Uni I (n = 9), and with *z* for the group Uni II (n = 11). For further explanation of the patient groups Uni I and Uni II see Table 3

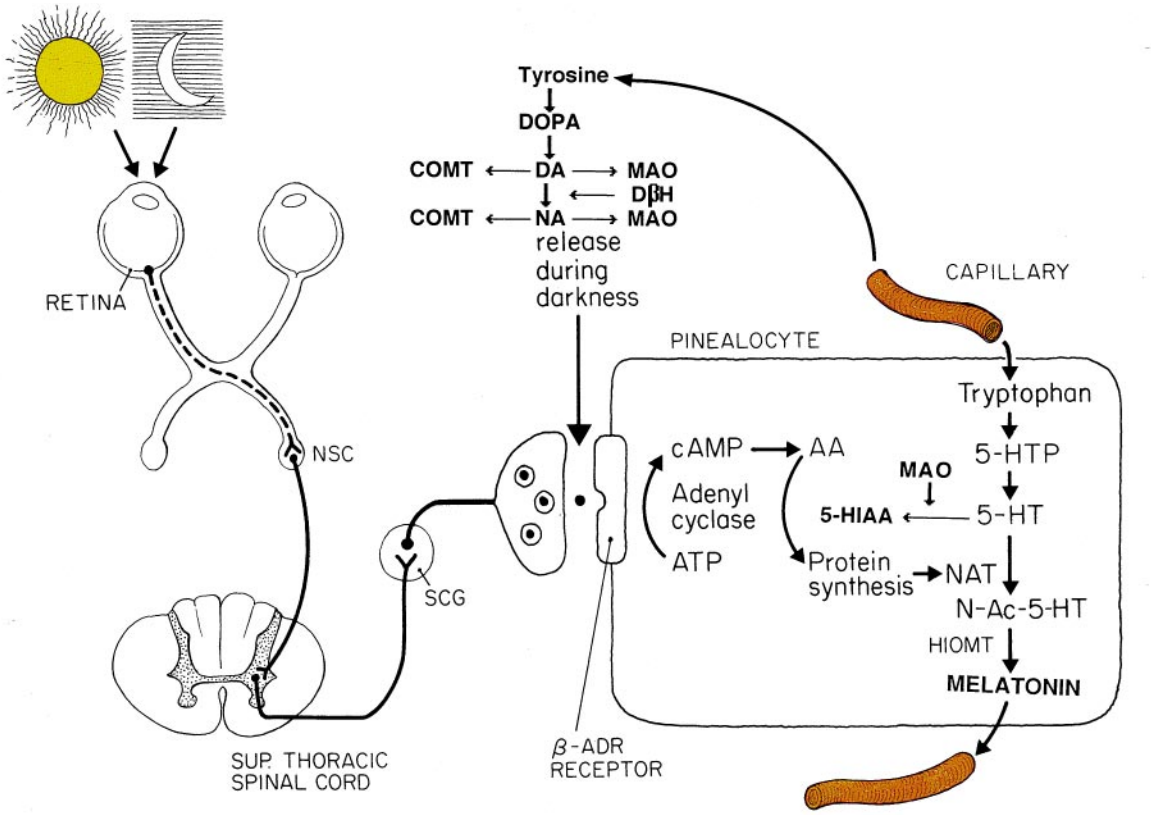

Fig. 4 The interrelationship between the tyrosine and tryptophan metabolic pathways, partly regulated by the neuroenzymes catechol-O-methyltransferase (*COMT*), dopamine-beta-hydroxylase (*DβH*) and monoamine oxidase (*MAO*), and the melatonin-rhythm regulating system, which includes the eye, hypothalamus and neural pathway to the pinealocytes. Variations in the environmental light–dark cycle regulate the protein synthesis of N-acetyltransferase (*NAT*) via noradranaline (*NA*) acting on beta-adrenergic receptors. The neurotransmitter serotonin (*5-HT*) is sequentially converted to N-acetyl-5-hydroxy-tryptamine (N-Ac-5-HT) by NAT, and the melatonin by hydroxyindole-O-methyltransferase (*HIOMT*) in the pineal gland. 5-HT may also be metabolised by the MAO enzyme. In the metabolic pathway, seen in the upper part of the figure, tyrosine is transformed to dopamine (DA) via DOPA and further acted on by DβH to form noradrenaline. DA and NA are both catabolised by COMT as well as by MAO. *NSC* Suprachiasmatic nuclei, *SCG* superior cervical ganglia, *cAMP* cyclic adenosine monophosphate, *ATP* adenosine triphosphate, *5-HTP* 5-hydroxy-tryptophan

Beck-Friis (1983) found an inverse association between 24-h serum melatonin and serum cortisol, and postulated the existence of a low-melatonin syndrome in some patients with unipolar depression. Support for an inhibitory-state-dependent effect of steroids on melatonin production in the pineal gland has been reported by Yuwiler (1989) who found that glucosteroids decrease the rise in pineal serotonin N-acetyltransferase activity upon stimulation with beta agonists. Another interesting relationship between steroid hormones and melatonin production has recently been reported by Holsboer and his research group. They found that administration of corticotropin-releasing hormone (CRH) to humans had an inhibitory effect on the production of melatonin (Kellner et al. 1997). The finding that CRH inhibits melatonin secretion and the mutual feedback between the HPA system and the pineal gland may increase understanding neuroendocrine alterations during the adaptive responses to different types of stressors.

As shown in Fig. 4, melatonin concentration could serve as a marker for noradrenergic tone as well as for serotonin (5-HT) function in the central nervous system, or at least in the pineal gland. If low melatonin concentrations reflect a deficiency in noradrenaline at receptor sites, then those with low melatonin should respond to noradrenergic antidepressants.

This can be tested. A dysfunction in the serotonergic system could also cause the low-melatonin syndrome in depression. The metabolic interrelationship between MAO and melatonin is illustrated in Fig. 4. Possible regulatory dysfunctions involve several processes in the metabolic pathways of the neuroenzymes N-acetyl-serotonin-transferase (NAT) and hydroxyindole-O-methyl transferase (HIOMT). As mentioned previously, NAT activity is altered by glucocorticoids, but in the same experiment steroid exposure of pineal glands did not affect HIOMT enzyme activity (Yuwiler 1989).

The amino acids, tyrosine and tryptophan, which are precursors of the three main neurotransmitters, noradrenaline, 5-HT and dopamine (DA), are believed to be important in the aetiology of affective disorders and are metabolically connected at the pineal level. The neuroenzymes catechol-O-methyltransferase (COMT) and dopamine-beta-hydroxylase (DBH) may also be influenced by different activity levels of MAO. In depression, insufficient

or excess activity of MAO may cause disturbed feed back regulatory mechanisms in the other enzymes involved in the neurotransmitter pathways at several metabolic steps, as shown in Fig. 4.

In a chronobiological sense, health can be defined as the individual's ability to maintain well-functioning rhythms, i.e. to adapt the external and internal biological rhythms which are partly regulated by variation in the light–dark cycle. The melatonin-rhythm regulating system, which includes the eye, hypothalamus and neural pathway to the pinealocytes, may serve as a regulator of the body's biological rhythms (Fig. 4).

Variations in the environmental light–dark cycle regulate the protein synthesis of N-acetyl-5-HT-transferase (NAT) via noradrenaline and beta-adrenergic receptors. In depressed patients melatonin seems to be involved in this adaptation. In depressed patients insufficient or excess melatonin production may therefore impair this adaptation with a subsequent locked rhythmic capacity manifest in a persistence of dysphoric mood and prolonged depressive episodes.

Some evidence against a generalised reduction in beta-adrenergic receptors in depression has recently been reported by Little et al. (1997) who found no difference in beta receptor numbers in postmortem pineal glands from a small population of 7 patients and 7 age- and gender-matched controls. Canadian investigators have recently reported that melatonin could be a trait marker in bipolar affective disorder (Kennedy et al. 1996). The heritability of melatonin production has been estimated in a Swedish population to be 0.53, which means that this trait is highly heritable in humans (Wetterberg et al. 1983). In that study the putative existence of a major gene with effect on the trait was considered to explain 75% of the total phenotypic variation of melatonin.

The neurotransmitter serotonin is metabolised by both MAO and NAT. In the metabolic pathway, shown in the upper part of Fig. 4, tyrosine is transformed into dopamine via DOPA and further by DBH to noradrenaline. Dopamine and noradrenaline are both catabolised by COMT as well as by MAO. Another relationship between MAO and melatonin is that the melatonin-forming enzymes HIOMT and MAO, are both localised on chromosome X. MAO-A and MAO-B are localised tail to tail on the short arm of this chromosome, without any distance to allow cross-over, and HIOMT is situated on the long arm of the same chromosome.

The next questions related to the present study concern possible differences in pharmacological response, in genetic history and in genetic composition of the patient groups. Lim et al. (1995) have reported evidence for a genetic association between bipolar disorder and alleles at three MAO-A markers, but not with alleles of MAO-B polymorphism. The results were significant only in female patients. In a planned 20-year follow-up of the present study with further laboratory tests, the possible genetic association between the alleles of the MAO genes and the three clinical subpopulations, one bipolar and two unipolar, will be examined.

In summary, platelet MAO activity, serum melatonin and abnormal suppression of the dexamethasone challenge test, together with the results of clinical rating scores, were analysed by multivariate methods. Three clinical subpopulations were found, one with latent bipolar disorder and two with unipolar depression. Because of the limitations inherent in statistical methods, the results must be interpreted with caution so as to avoid misinterpretation. Replication studies are needed to refute, or verify, the reported findings of the three main subgroups of patients with affective disorders, particularly a population with latent bipolar illness.

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